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DOCUMENT-IDENTIFIER: US 7229810 B2
TITLE: Polymer conjugates of proteinases

PRIOR-PUBLICATION:

DOC-ID	DATE
US 20030012777 A1	January 16, 2003

Description Paragraph (35):
Functional assays include the measurement of catalytic activity using polypeptide or protein substrates for proteinases, preferably under standardized conditions. As used herein, "standardized conditions" for the measurement of the activity of Proteinase K are defined as incubation for 1 hour at 52.degree. C. of about 0.6 mcg/mL Proteinase K with 5.5 mg/mL casein (Sigma, catalog #C 7078) in 45 mM Tris-HCl buffer, pH 8. Proteinase K and the other subtilases belong to the class of enzymes known as serine proteinases, which contain a functional serine residue within the active site (Siezen R. J., et al., (1991) Protein Eng 4:719 737). Casein and hemoglobin are model substrates for such enzymes. The digestion of casein is referred to herein as "caseinolytic activity" and can be monitored spectrophotometrically or turbidimetrically, using methods known in the art (see Flambard, B., et al., (2000) Appl Environ Microbiol 66:5134 5140). Functional assays are useful for studying the effects of harsh conditions on enzymatic activity. Such conditions include exposure to elevated temperatures in the absence or presence of chaotropic agents. For example, urea, guanidinium salts and SDS are commonly used denaturing agents (Porteous, L. A., et al., (1994) Curr Microbiol 29:301 307; Goldenberger, D., et al., (1995) PCR Methods Appl 4:368 370; Hossain, A. M., et al., (1997) Mol Hum Reprod 3:953 956). Typical studies include the incubation of a sample of an unmodified or a derivatized proteinase for various periods at an elevated temperature in the presence or absence of a denaturant.

Description Paragraph (41):
The conjugates, compositions and kits of the present invention may be used in conjunction with any application involving the use of proteinases, particularly proteinases of the subtilisin family of serine proteinases, including Proteinase K. The conjugates, compositions and kits of the present invention will find particular application in methods of manipulating nucleic acid molecules, for example in industrial genomics methods employing the extraction and/or isolation of nucleic acid molecules. Such techniques may include, for example, nucleic acid isolation, amplification, synthesis, sequencing, fragment analysis and linear gene mapping. The conjugates, compositions and kits of the present invention will find particular application in methods for detection and identification of disease-related prion protein molecules, for example in the diagnosis of transmissible and hereditary spongiform encephalopathies. Such techniques may include, for example, high throughput screening of samples of tissue from animals intended for food, samples obtained from actual or prospective blood or organ donors and samples from patients at risk.

Other Reference Publication (5):
Bajorath, J., et al., "Autolysis and inhibition of proteinase K, a subtilisin-related serine proteinase isolated from the fungus Tritirachium album Limber,"

Biochim. Biohys. Acta 954:176-182, Elsevier Science B.V. (1988). cited by other

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